sequencing-based diversity studies. Nucleic Acids Res. 2013;41:e1. https://doi.org/10.1093/nar/gks808
7. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13:581–3. https://doi.org/10.1038/nmeth.3869
8. Mediannikov O, Sekeyová Z, Birg M-L, Raoult D. A novel obligate intracellular gamma-proteobacterium associated with ixodid ticks, *Diplorickettsia massiliensis*, gen. nov., sp. nov. PLoS One. 2010;5:e11478. https://doi.org/10.1371/journal.pone.0011478
9. Subramanian G, Mediannikov O, Angelakis E, Socolovschi C, Kaplanski G, Martzolff L, et al. *Diplorickettsia massiliensis* as a human pathogen. Eur J Clin Microbiol Infect Dis. 2012;31:365–9. https://doi.org/10.1007/s10096-011-1318-7

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Case of *Babesia crassa*–Like Infection, Slovenia, 2014

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We report a case of *Babesia crassa*–like infection in an asplenic patient in Slovenia in 2014. We diagnosed the infection using microscopy, 18S rRNA sequencing, and serology and monitored parasitemia using digital PCR. With its increasing occurrence, babesiosis should be included in differential diagnoses for immunocompromised patients displaying fever.

Babesia infections occur worldwide and cause disease mainly in animals, but disease occurs occasionally in humans. Infections in humans are mostly attributable to *B. microti*, *B. duncani*, and *Babesia* sp. MO1 in North America; *B. divergens*, *B. venatorum*, and *B. microti* in Europe; and *B. venatorum*, *B. crassa*–like parasite, *B. microti*, *Babesia* sp. XXB/HangZhou, and *Babesia* sp. KO-1 in Asia (*1*,*2*). Transmission occurs predominantly through tick bites, but humans have acquired infections via contaminated blood products and through the transplacental and perinatal routes (*1*). Most patients with *Babesia* infections in Europe were reported to be asplenic or immunocompromised. Typical clinical signs and symptoms include fever (up to 40°C), parasitemia (20%–80%), severe anemia, muscle weakness, fatigue, and late-onset jaundice with dark urine, and sometimes complications develop. Long-term clinical follow-up that includes blood smear examination and PCR analysis is necessary because relapse and persistence of parasitemia can occur in spite of treatment. The application of novel molecular methods has revealed that the host range of many *Babesia* species is less restricted than previously thought. New species or animal pathogens are increasingly being discovered as causing *Babesia* infections in humans. We report a *B. crassa*–like infection in a patient in Slovenia in 2014.

In 2014, a 55-year-old woman, living on the outskirts of Murska Sobota, Slovenia, sought medical treatment for a 6-day history of intermittent fever up to 39°C, myalgia, headache, poor appetite concomitant with weight loss, fatigue, sweating, and dark urine. She previously had a splenectomy and partial pancreatectomy 5 years previous because of cystic adenoma and adrenal incidentaloma without hormonal activity. She reported no history of travel, tick bite, animal contact, or blood transfusions.

Her blood pressure was 115/70 mm Hg, heart rate 83 beats/min, and body temperature 36.6°C, and a physical examination indicated no significant clinical findings. The first basic blood analysis revealed thrombocytopenia (platelets 85 × 10^9/L). A differential blood analysis indicated that the concentration of large unstained cells was elevated (0.41 × 10^9/L, reference range 0–0.4 × 10^6/L). Biochemical laboratory testing showed mild fluctuations in liver functioning: aspartate aminotransferase 1.22 (reference range 0.17–0.51) μkat/L, alanine aminotransferase 1.13 (reference range 0.17–0.68) μkat/L, γ-glutamyltransferase 1.08 (reference range 0.03–0.51 μkat/L) μkat/L, and alkaline phosphatase 1.88 (reference range 0.5–2.0) μkat/L. C-reactive protein was 51 mg/L (150 [reference range 0.76–28.5] nmol/L), and mild erythrocyturia was present. Giemsa-stained blood smears showed unusual inclusions in erythrocytes, Howell-Jolly bodies, mild anisocytosis, some atypical lymphocytes, and some large thrombocytes. We observed many ring forms and some paired piriform shapes of *Babesia* spp. in blood smears (Figure), and parasitemia was 1% (Appendix Table,

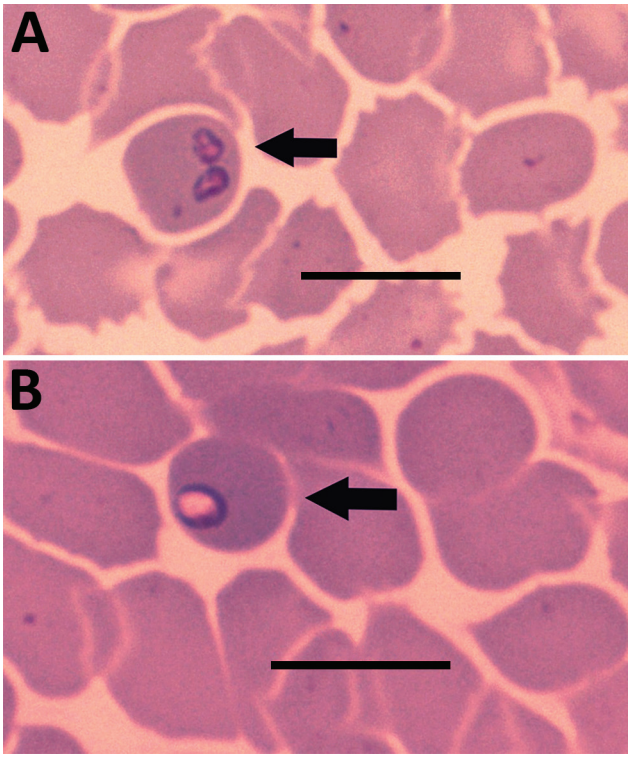

Figure. Piriform (A) and ring shapes (B) in blood smear of sample taken from patient with *Babesia crassa*–like infection, Slovenia, 2014. Smear was Wright-Giemsa stained. Scale bars indicate 50 µm.

https://wwwnc.cdc.gov/EID/article/26/5/19-1201-App1.pdf). We confirmed diagnosis by conventional PCR and sequencing of the 18S rRNA gene (*3*). A phylogenetic analysis indicated the pathogen was the *B. crassa*–like parasite (Appendix Figure).

We gave the patient an oral treatment of clindamycin (600 mg 3×/d) and quinine (600 mg 3×/d). Three days later, the patient was normothermic, and after a total of 6 days, she was discharged from the hospital with platelet levels within the reference range (150–350 × 10^9/L). She continued the dual therapy for 14 days. To follow up on the patient's response to treatment, we measured parasitemia levels by blood smear microscopy, PCR (*3*), and digital PCR (Appendix).

We later confirmed the infection by serology using an indirect immunofluorescence assay specific to another member of the large *Babesia* group, *B. divergens* (MegaFLUO BABESIA divergens; Megacor, https://www.megacor.at). Antibodies were cross-reactive, and results demonstrated a 4-fold increase in IgG titer (Appendix Table).

Reports of babesiosis in humans are increasing with the increase in number of immunocompromised persons; a species previously known only as an animal pathogen is posing a greater threat to those with weakened immune systems. *B. crassa* has been detected in sheep in Iran (*4*), goats and ticks in Turkey (*5*,*6*), and ticks in Hungary (*7*), and a case series of infections with *B. crassa*–like parasite in humans, sheep, and ticks was reported in northeastern China (*8*).

We report an infection of *B. crassa*–like parasite in an asplenic person in Europe that was confirmed by blood smear examination, PCR, sequencing, and serology (with assay specific to distant relative *B. divergens*). The patient recovered after treatment with the standard dual antimicrobial regimen. In addition to blood smear, we used a unique digital PCR assay to follow the decrease in concentration of babesial DNA in the patient's blood until complete recovery. Note that DNA levels in blood do not necessarily correlate with levels of live pathogen (i.e., active infection).

With the development of new and more sensitive diagnostic techniques, parasites like *Babesia* spp., primarily recognized as animal pathogens, are becoming increasingly reported as human pathogens too, even in areas where the parasite has not been reported previously. Babesiosis should be included in the differential diagnoses for immunocompromised patients displaying fever worldwide.

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References

1. Hildebrandt A, Gray JS, Hunfeld K-P. Human babesiosis in Europe: what clinicians need to know. Infection. 2013; 41:1057–72. http://dx.doi.org/10.1007/s15010-013-0526-8
2. Krause PJ. Human babesiosis. Int J Parasitol. 2019;49:165–74. http://dx.doi.org/10.1016/j.ijpara.2018.11.007
3. Olmeda AS, Armstrong PM, Rosenthal BM, Valladares B, del Castillo A, de Armas F, et al. A subtropical case of human babesiosis. Acta Trop. 1997;67:229–34. http://dx.doi.org/10.1016/S0001-706X(97)00045-4
4. Schnittger L, Yin H, Gubbels MJ, Beyer D, Niemann S, Jongejan F, et al. Phylogeny of sheep and goat *Theileria* and *Babesia* parasites. Parasitol Res. 2003;91:398–406. http://dx.doi.org/10.1007/s00436-003-0979-2

5. Ozubek S, Aktas M. Molecular evidence of a new *Babesia* sp. in goats. Vet Parasitol. 2017;233:1–8. http://dx.doi.org/10.1016/j.vetpar.2016.11.016
6. Orkun Ö, Karaer Z. Molecular characterization of *Babesia* species in wild animals and their ticks in Turkey. Infect Genet Evol. 2017;55:8–13. http://dx.doi.org/10.1016/j.meegid.2017.08.026
7. Hornok S, Takács N, Kontschán J, György Z, Micsutka A, Iceton S, et al. Diversity of *Haemaphysalis*-associated piroplasms of ruminants in Central-Eastern Europe, Hungary. Parasit Vectors. 2015;8:627. http://dx.doi.org/10.1186/s13071-015-1236-0
8. Jia N, Zheng YC, Jiang JF, Jiang RR, Jiang BG, Wei R, et al. Human babesiosis caused by a *Babesia crassa*-like pathogen: a case series. Clin Infect Dis. 2018;67:1110–9. http://dx.doi.org/10.1093/cid/ciy212

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Hepatitis A Hospitalization Costs, United States, 2017

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The United States is in the midst of unprecedented person-to-person hepatitis A outbreaks. By using Healthcare Cost and Utilization Project data, we estimated the average costs per hepatitis A–related hospitalization in 2017. These estimates can guide investment in outbreak prevention efforts to stop the spread of this vaccine-preventable disease.

The introduction of hepatitis A vaccine has dramatically changed the epidemiology of hepatitis A in the United States. After vaccine licensure in 1995, hepatitis A incidence declined substantially; 3,366 hepatitis A cases were reported nationally in 2017 (*1*).

During July 1, 2016–February 7, 2020, state health departments publicly reported >31,000 outbreak-associated cases, primarily affecting persons who use drugs and persons experiencing homelessness, in the largest person-to-person hepatitis A outbreaks in the postvaccine era (*2*). More than 18,900 (61%) outbreak-associated patients have reportedly been hospitalized in these outbreaks (*2*). As these unprecedented outbreaks continue, we sought to estimate the average direct medical costs per hepatitis A–related hospitalization, which can be used to guide investment in outbreak prevention efforts.

We analyzed data from the 2017 Healthcare Cost and Utilization Project National Inpatient Sample (NIS). NIS, a 20% stratified sample of discharges from US community hospitals, is the largest publicly available all-payer inpatient database in the country (*3*). We considered a hospitalization to be hepatitis A–related if it included codes B15.0 or B15.9 from the International Classification of Diseases, 10th Revision, Clinical Modification, as any of the 30 listed diagnosis codes. We converted the total hospital charges into cost estimates (in 2017 US dollars) by multiplying total charges with 2017 hospital-specific cost-to-charge ratios (*4*), then estimated the average cost of hospitalization, SD, and 95% CI on the basis of the NIS survey sampling design. We multiplied the average costs by the number of patients hospitalized for outbreak–associated hepatitis A to generate an estimate of the preventable economic burden of hospitalizations in the ongoing person-to-person outbreaks (*2*).

We examined hepatitis A–related hospitalizations in the 2017 NIS dataset for evidence of associated liver transplantation (procedure codes 0FY00Z0, 0FY00Z1, and 0FY00Z2 from the International Classification of Diseases, 10th Revision, Clinical Modification, listed as any of the 15 procedure codes). Because the unweighted number of hospitalizations associated with liver transplantation was <10, we included such hospitalizations in the analysis but did not report them as a separate category (*5*).

Overall, the average costs per hepatitis A–related hospitalization in the United States in 2017 were $16,232 (SD $602; 95% CI $15,052–$17,411). The average costs ranged from $12,921 (SD $1,443; 95% CI $10,091–$15,750) in the West North Central Census Division to $19,680 (SD $1,932; 95% CI $15,891–$23,467) in the Pacific Census Division.

During July 1, 2016–February 7, 2020, a total of 32 states reported >18,900 outbreak-associated hepatitis A hospitalizations resulting from the ongoing hepatitis A outbreaks (*2*). On the basis of results of our analysis as a multiplier, we estimate that hospitalization costs associated with these outbreaks have exceeded $306.8 million (SD $11.4 million) as of February 7, 2020.

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Appendix

To follow up a patient's response to the treatment we measured the level of parasitemia with blood smear using microscopy, conventional PCR (*1*) and digital PCR (dPCR) (Appendix Table). While peripheral blood smears were positive only until 5th day of hospitalization, DNA was detected for one and a half months (Appendix Table). At the last follow-up visit, three months later, both conventional PCR, dPCR and blood smear were negative. The second day after beginning of the treatment the parasitemia started to decrease. The blood smear was negative after five days, where DNA concentration decreased for 1.6 log (Appendix Table). In the following days a consistent, but slow decrease of DNA was demonstrated until the patient became completely negative three months later (Appendix Table). By using digital PCR, we have demonstrated that novel molecular techniques are more sensitive than direct blood smear microscopy or conventional PCR.

PCRs, Sequencing and Phylogenetic Analysis of a Part of Genome of *Babesia* spp.

To investigate which species is responsible for the patient's disease we first sequenced the PCR (*2*) product (353 bp) of 18S rRNA gene of *Babesia* spp. After analysis the sequence of the product was compared with previously published sequences deposited in GenBank by BLAST. We retrieved the 99% identity with sequence of *Babesia* spp. AM-HC344 from a tick *Haemaphysalis concinna* from Russia (Far East, Amur Region; GenBank Acc. No. KJ486564). As a next step we performed a conventional PCR on a nearly complete 18S rRNA gene with primers CryptoF and CryptoR (*3*). Approximately 1700 bp long product was sequenced with additional sequencing primers (*3*). After the comparison of a sequence of the product with published sequences we retrieved 98% identity with *Babesia crassa* from sheep in Iran (Acc. No.

AY260176). The sequence of a nearly complete 18S rRNA gene of *B. crassa*-like babesia from a Slovenian patient is deposited in the GenBank under Acc. No. MK240324.

To confirm the result of a sequencing of 18S rRNA gene we performed additional conventional PCR amplifying a partial segment of a beta-tubulin gene (748 bp) of *B. crassa*–like (*1*) pathogen. After sequencing with additional sequencing primers on both strands, analysis and comparison with sequences in GeneBank we retrieved 99% identity with a sequence of *B. crassa*-like (Acc. No. KX827593) from a tick in China. The sequence of 18S rRNA gene of *B. crassa*-like babesia from a Slovenian patient is deposited in the GenBank under Acc. No. ML230987.

In a phylogenetic tree of partial 18S rRNA gene a sequence of a babesia from our patient clustered together with *B. crassa* – like sequences from ticks from China and from sheep from Iran and Turkey (Appendix Figure).

Quantification of Parasitemia with Digital PCR

For quantification of *Babesia crassa* – like pathogen we have developed a digital PCR (dPCR) on QuantStudio™ 3D Digital PCR System (Thermo Fisher Scientific, Applied Biosystems, USA). The Primer Express Software v3.0.1 (Thermo Fisher Scientific, Applied Biosystems, USA), with default conditions, was used for designing primers Bab_Irk_F1 (5'-CTA GCT GTC GAG AGA TAG TTT CGA CT-3'), Bab_Irk_R1 (5'-GCA TCA CAG ACC TGT TAT TGC CTT-3') and probe Bab_Irk_P1 (5'-6FAM-AGA GGG ACT CCT GTG CGT CAA GCG TAG GGG-BHQ1-3') targeting 94 base pair (bp) long hypervariable region of *Babesia* spp. 18S rRNA. Specificity of developed primers and probe was checked with BLAST.

For the QuantStudio™ 3D digital PCR system, the reactions were prepared in a final volume of 15 µL, composed of: 7.5 µL of Mastermix QuantStudio® 3D Digital PCR, 0.3 µL of Bab_Irk_F1 (50 µM), 0.3 µL of Bab_Irk_R1 (50 µM), 0.2 µL of Bab_Irk_P1 (20 µM), 5.2 µL of water and 1.5 µL of DNA at different dilutions. Results were expressed as DNA copies per µL. The reaction mix was loaded onto the QuantStudio 3D digital PCR chips by using QuantStudio 3D digital PCR chip loader. The amplification conditions were: 95 °C for 20 sec, cycling conditions: 95 °C for 3 sec, 58 °C for 3 sec, 60 °C for 30 sec (40 cycles). The amplifications were performed in a Proflex™ 2x Flat PCR System. The chips were transferred to the

QuantStudio 3D Instrument for imaging. Data elaboration was executed using the cloud-based QuantStudio 3D Analysis Suite software (version 3.0.03) in the absolute quantification module maintaining automatic settings. For each run, at least one negative control was included. The quality threshold was set at the default value of 0.5, to define the accepted wells and ranged from 13,265 to 21,912 with a mean of 17,136.

References

1. Jia N, Zheng YC, Jiang JF, Jiang RR, Jiang BG, Wei R, et al. Human babesiosis caused by a *Babesia crassa*-like pathogen: a case series. Clin Infect Dis. 2018;67:1110–9. PubMed http://dx.doi.org/10.1093/cid/ciy212

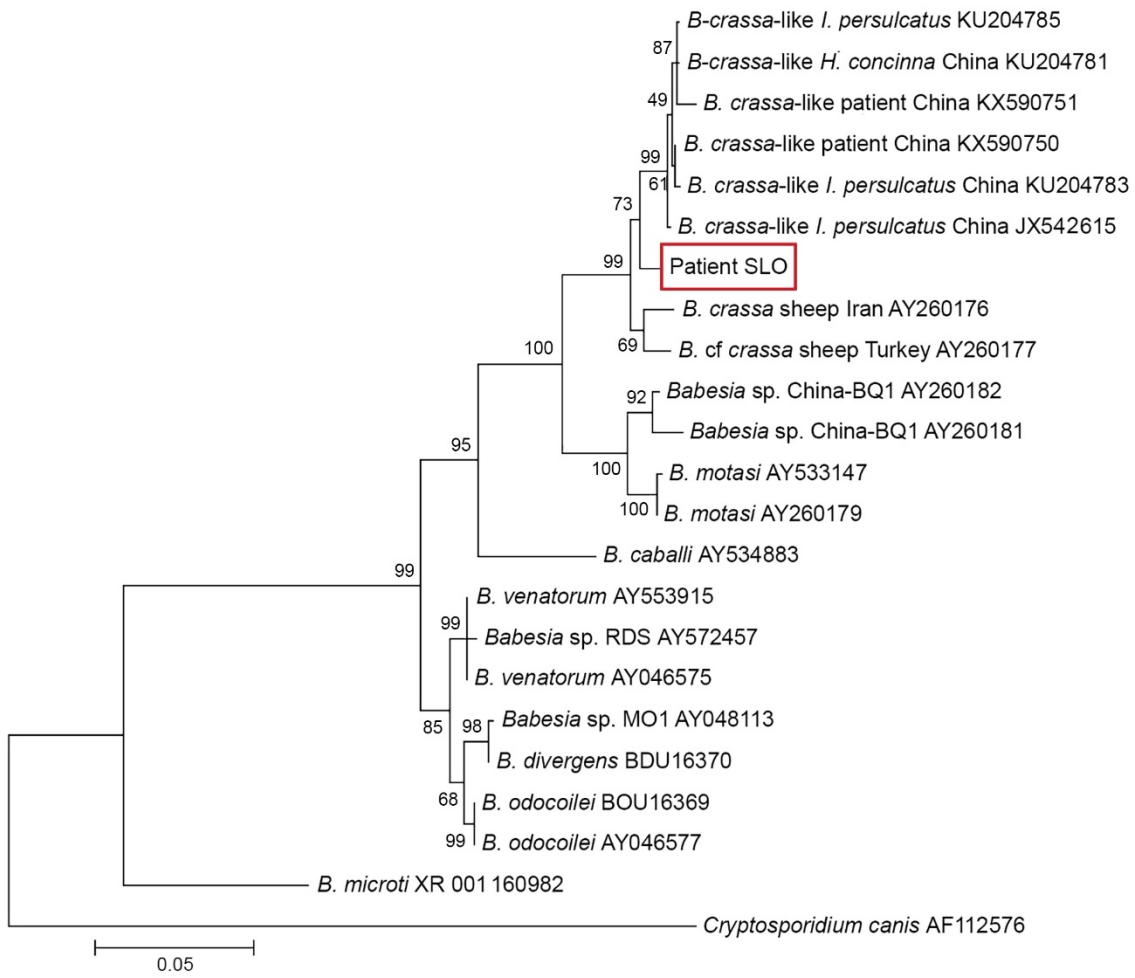
2. Olmeda AS, Armstrong PM, Rosenthal BM, Valladares B, del Castillo A, de Armas F, et al. A subtropical case of human babesiosis. Acta Trop. 1997;67:229–34. PubMed http://dx.doi.org/10.1016/S0001-706X(97)00045-4

3. Holman PJ, Madeley J, Craig TM, Allsopp BA, Allsopp MTEP, Petrini KR, et al. Antigenic, phenotypic and molecular characterization confirms *Babesia odocoilei* isolated from three cervids. J Wildl Dis. 2000;36:518–30. PubMed http://dx.doi.org/10.7589/0090-3558-36.3.518

Appendix Table. Results of microbiological tests of blood samples from day one to complete recovery (day 113)*

Day of visit/hospitalization	Blood smear (%)	Parasitemy – dPCR result (log DNA/ml)	IIF IgG (titer)
1st	1.0	10.8	1:32
2nd†	NA	10.7	
3rd	0.6	10.1	
4th	0.4	10.0	
5th	0.2	9.3	
6th	negative	9.5	
8th	negative	8.4	1:512
9th	negative	7.9	
13th‡	negative	7.5	
23th	negative	7.1	1:512
35th	NA	6.7	
49th	NA	6.1	1:512
90th	negative	5.0§	1:512
105th	negative	negative	
113th	negative	negative	

*dPCR, digital PCR; IIF, indirect immunofluorescence; NA, not available.
†start of treatment.
‡end of treatment after fourteen days.
§conventional PCR negative.



Appendix Figure. A phylogenetic tree of partial 18S rRNA gene sequences of *Babesia* spp. using Neighbour – joining method. The tree is rooted using *Cryptosporidium canis* as an outgroup. The bootstrap values based on 1000 replicates are displayed next to the branches. Sequences of babesiae and *Cryptosporidium sp.* and corresponding accession numbers were retrieved from GeneBank on January 2019. Accession number of partial 18S rRNA gene sequence of *Babesia* spp. from Slovenian patient is MK240324. SLO, Slovenia.